

## Annual Progress Report

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Institution: University of Iowa

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Objective: To examine the mechanisms by which melanopeptides and hypertonic saline activate pressor mechanisms during hemorrhagic shock.

Approach: Hemorrhagic shock is induced in rats by the controlled withdrawal of specific blood volumes, graded by body weight. Mean arterial pressure (MAP) and heart rate (HR) serve as indices of cardiovascular function, and ultimately as indicators of hemorrhage-induced shock. In this model of hemorrhagic shock, the melanopeptide,  $\gamma$ -MSH, is administered peripherally or centrally, post-hemorrhage, to determine the therapeutic potential of the compound in treating hemorrhagic shock. The ability of  $\gamma$ -MSH and hypertonic saline to increase survivability under the condition of hemorrhagic shock is being evaluated.

Accomplishments (last six months): The primary objective of our work has been to examine the cardiovascular characteristics of hemorrhage-induced hypotension and shock. Our knowledge of these conditions will allow us to investigate the therapeutic efficacy of the sympathoexcitatory agents,  $\gamma$ -MSH and hypertonic saline.

Previously (report submitted for 1 January 1997 – 15 July 1997) we examined the ability of  $\gamma$ -MSH to increase the physiological compensation for moderate hemorrhage-induced hypotension. The actions of  $\gamma$ -MSH compared to control animals were manifested in increased MAP and HR. These findings suggest a role for  $\gamma$ -MSH in the treatment of moderate hemorrhage-induced hypotension.

We have now expanded our work to develop a protocol dealing directly with advanced irreversible hemorrhagic shock. This model will address the ability of  $\gamma$ -MSH to promote physiological compensation thereby preventing one from reaching irreversible hemorrhagic shock. The materials and methods for the following experiments were identical to those stated in the attached July 15, 1997 progress report. Previously, the withdrawal of 2.6 and 2.8 ml per 100g body weight produced a sub-lethal hypotension resulting in a 50% decrease in MAP. We have now systematically increased the blood volume removed during controlled hemorrhage. The purpose for this was to 1) develop a cardiovascular profile for different levels of hemorrhage, and 2) determine a hemorrhage volume that produced 100% mortality.

Adult male rats weighing approximately 300-325 grams were instrumented with two arterial catheters and a venous catheter. One arterial catheter in the carotid artery was used for blood withdrawal and the other arterial catheter located in the femoral artery was used for blood pressure recording. The venous catheter was used for drug administration. This combination of catheters allowed for injections to be made without handling the animals and blood to be withdrawn while continuous recording of blood pressure was maintained. Following catheterization, animals were allowed 48 hours recovery and then tested for their blood pressure and HR responses to hemorrhage.

Rats were hemorrhaged at 2.8, 3.0, 3.2, 3.6, 3.8 and 4.0 ml per 100g body weight and were observed for 90 minutes post-hemorrhage. The rats were hemorrhaged at 1 ml per minute. MAP and HR were recorded. The MAP and HR data are summarized in Tables 1 and 2, respectively, and are illustrated in Figures 1 and 3, respectively. These data show that hemorrhage volumes from 2.8 to 3.6 ml per 100g of body weight, while producing a marked hypotension, were not lethal. However, hemorrhage volumes of 3.8 and 4.0 ml per 100g body weight produced 100% mortality within the 90-minute observation period.

Next, we examined the effects of  $\gamma$ -MSH infusions (0.825  $\mu\text{g}/\text{min.}$  & 4.125  $\mu\text{g}/\text{min.}$  over 1 hour) administered post 3.8 ml per 100g body weight hemorrhage, on mortality. The MAP and HR data are summarized in Tables 1 and 2, respectively, and are illustrated in Figures 2 and 4, respectively. Survival data is illustrated in Figure 5. Both  $\gamma$ -MSH infusions produced increases in MAP and HR. We observed 100% mortality in the 3.8ml/100g hemorrhage group that did not receive the  $\gamma$ -MSH infusion (Fig. 5). However, there was a decrease in mortality in both groups that received the infusion of  $\gamma$ -MSH (0.825  $\mu\text{g}/\text{min.}$  & 4.125  $\mu\text{g}/\text{min.}$  over 1 hour) (Fig. 5). These data suggest that  $\gamma$ -MSH promotes compensation and therefore survivability under irreversible hemorrhagic conditions, which in control animals produced 100% mortality.

Significance: Our work provides evidence that the administration of  $\gamma$ -MSH under conditions of hemorrhagic shock may decrease mortality. Now that we have established a working model of hemorrhagic shock we can employ this protocol to further and more completely characterize the pharmacodynamics of both  $\gamma$ -MSH and hypertonic saline under these conditions.

Work Plan (Next 12 months): Employing our protocol for irreversible hemorrhagic shock, we plan to further characterize the actions of  $\gamma$ -MSH and hypertonic saline. This will include testing both peripheral and central administration of these compounds. We also plan to expand the range of doses administered, as well as forms of peripheral administration (bolus, infusion or a combination of the two). We also plan to investigate the effects of combined therapy ( $\gamma$ -MSH and hypertonic saline). Another aspect of this research will look into the hemodynamic effects of  $\gamma$ -MSH and hypertonic saline through the direct measurement of blood flow.

Publications (1 Jan 97 - 31 Dec 97):

- Johnson, R. F., & Johnson, A. K.: The interaction of meal-related, rhythmic and homeostatic mechanisms and the generation of thirst and drinking. Brazilian Journal of Medical and Biological Research, 1997, 30, 487-491.
- Fuchs, L. C., Landas, S. K., & Johnson, A. K.: Behavioral stress alters coronary vascular reactivity in borderline hypertensive rats. Journal of Hypertension, 1997, 15, 301-307.
- Davisson, R. L., Travis, M. D., Bates, J. N., Johnson, A. K., & Lewis, S. J.: Stereoselective actions of S-nitrocysteine in the central nervous system of conscious rats. American Journal of Physiology, 1997, 272, H2361-H2368.
- Johnson, A. K., & Thunhorst, R. L.: The neuroendocrinology of thirst and salt appetite: Visceral sensory signals and mechanisms of central integration. Frontiers in Neuroendocrinology, 1997, 18, 292-353.
- Muntzel, M. S., Thunhorst, R. L., & Johnson, A. K.: Effects of subfornical lesions on sympathetic nerve responses to insulin. Hypertension, 1997, 29, 1020-1024.
- Stauss, H. M., Persson, P. B., Johnson, A. K., & Kregel, K. C.: Frequency response characteristics of autonomic nervous system function in conscious rats. American Journal of Physiology, 1997, 273, H786-H795.
- Xu, Z., & Johnson, A. K.: Central renin injections: effects on drinking and expression of immediate early genes. Brain Research, (in press).
- Menani, J. V., De Luca, Jr., L. A., & Johnson, A. K.: Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by sodium depletion. American Journal of Physiology, (in press).
- Johnson, A. K.: Circumventricular organs. In: G. Adelman and B. Smith (eds.), Encyclopedia of Neuroscience. Amsterdam: Elsevier, (in press).
- Scrogin, K. E., Veelken, R., & Johnson, A. K.: Central methysergide prevents renal sympathoinhibition and bradycardia during hypotensive hemorrhage. American Journal of Physiology, (in press).

Table 1. Summary of MAP (mmHg) w/ SE

	<u>Baseline</u>	<u>5</u>	<u>10</u>	<u>20</u>	<u>30</u>	<u>40</u>	<u>50</u>	<u>60</u>	<u>70</u>	<u>80</u>	<u>90</u>
2.8 ml/100g											
MAP	123.02	77.36	78.8	72	95.14	93.65	95.52	94	93.84	96.73	95.23
SE	6.88	16.87	16.05	10.9	4.39	4.55	2.99	5.6	5.03	4.78	4.39
3.0 ml/100g											
MAP	120.39	84.33	100.11	65.07	86.87	91.03	87.4	80.75	83.32	86.79	90.37
SE	8.68	27.4	19.58	11.27	7.05	9.91	10.33	8.26	8.07	9.16	10.07
3.2 ml/100g											
MAP	134.3	116.54	49.43	95.43	105.98	106.48	105.6	101.06	101.34	100.85	101.4
SE	9.89	3.9	5.93	2.62	2.66	4.67	5.29	5.7	5.84	6.05	5.99
3.6 ml/100g											
MAP	129.54	126.72	100.18	65.52	81.9	95.12	88.59	83.89	86.01	88.83	90.29
SE	5.39	6.02	8.79	12.06	9.63	2.82	5.74	13.45	7.97	7.6	6.42
3.8 ml/100g											
MAP	125.2	101.33	58.17	71.06	57.09	74.47	70.6	51.33	55.86		
SE	2.6	9.93	3.66	3.21	10.1	1.56	2.95	26.08	0		
4.0 ml/100g											
MAP	134.07	102.22	62.46	59.68	60.18	47.67	23.23				
SE	8.83	20.64	10.78	6.08	11.57	18.64	19.21				
3.8 ml/100g inf. 0.825 micrograms/ml/min over 60min.											
MAP	127.8	93.41	72.64	65.89	88	88.78	88.15	89.32	87	82.55	79.15
SE	3.9	8.35	14.44	8.04	2.71	7.87	0	0	0	0	0
3.8 ml/100g inf. 4.125 micrograms/ml/min over 60min.											
MAP	125.49	108.61	61.46	94.42	64.43	86.19	84.57	88.23	84.44	76.25	73.53
SE	4.67	12.73	13.37	7.26	11.58	0.13	3.09	0.23	0.31	0.71	0.77

Table 2. Summary of HR (bpm) w/ SE

	Baseline	5	10	20	30	40	50	60	70	80	90
2.8 ml/100g											
mean HR	392.81	327.6	360.63	361.78	371.32	391.4	389.58	392.76	398.86	394.79	415.94
SE	18.53	39.1	33.48	38.5	27.02	28.12	23.04	21.98	27.37	31.59	26.27
3.0 ml/100g											
mean HR	420.14	362.89	456.57	375.79	419.5	427.97	431.6	432.08	418.41	410.61	414.2
SE	20.85	40.87	38.29	39.18	17.16	14.91	11.15	15.25	15.79	14.38	17.2
3.2 ml/100g											
mean HR	413.84	430.8	378.33	442.67	470.53	468.51	463.58	472.95	470.94	473.95	470.92
SE	21.83	19.56	28.52	12.6	5.74	2.22	1.43	7.26	9.66	11.55	10.71
3.6 ml/100g											
mean HR	367.39	366.39	392.64	409.01	390.61	415.05	431.44	437.89	421.75	419.39	415.65
SE	15.89	15.3	35.5	41.54	43.19	30.47	15.85	18.72	14.61	10.93	8.94
3.8 ml/100g											
mean HR	378.53	380.19	347.16	466.07	456.12	451.02	438.26	392.52	386.64		
SE	6.92	21.84	19.61	23.81	3.36	16.67	1.27	29.34			
4.0 ml/100g											
mean HR	398.03	372.25	336.31	472.29	467	438.09	329.09				
SE	28.63	57.4	53.4	37	16.73	24.49	76.32				
3.8 ml/100g inf. 0.825 micrograms/ml/min over 60min.											
mean HR	386.22	384.99	392.73	486.39	428.83	437.56	432.11	421.92	416.9	436.29	448.84
SE	19.46	33.78	52.5	13.61	6.83	4.92	0	0	0	0	0
3.8 ml/100g inf. 4.125 micrograms/ml/min over 60min.											
mean HR	370.39	392.42	375.47	475.81	442.69	418.97	416.68	408.04	405.34	397.19	386.06
SE	18.06	18.98	16.45	14.47	8.7	8.25	0.92	4.93	6.05	5.97	8.54

Figure 1. MAP change to hemorrhage

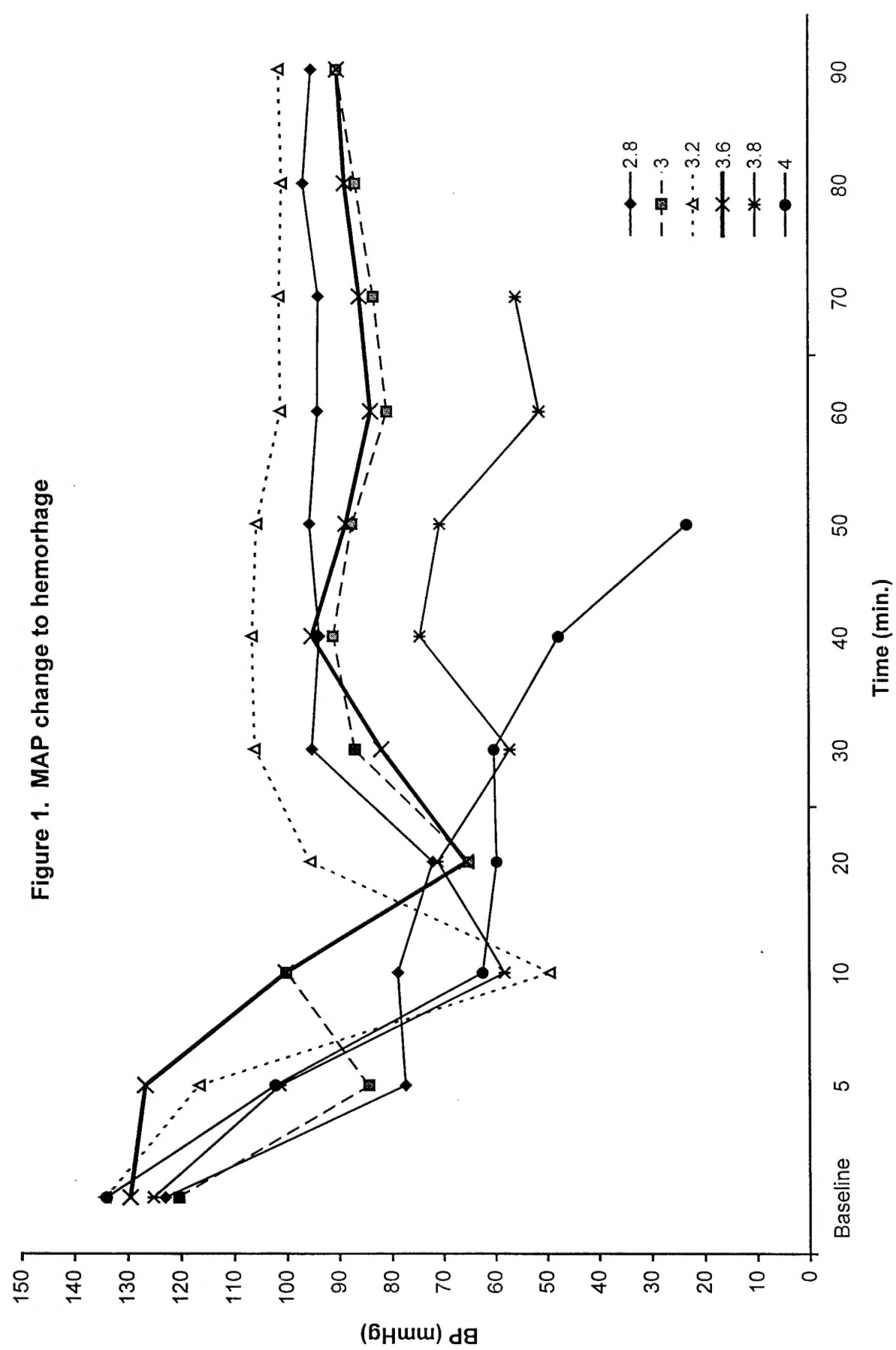


Figure 2. MAP change to hemorrhage

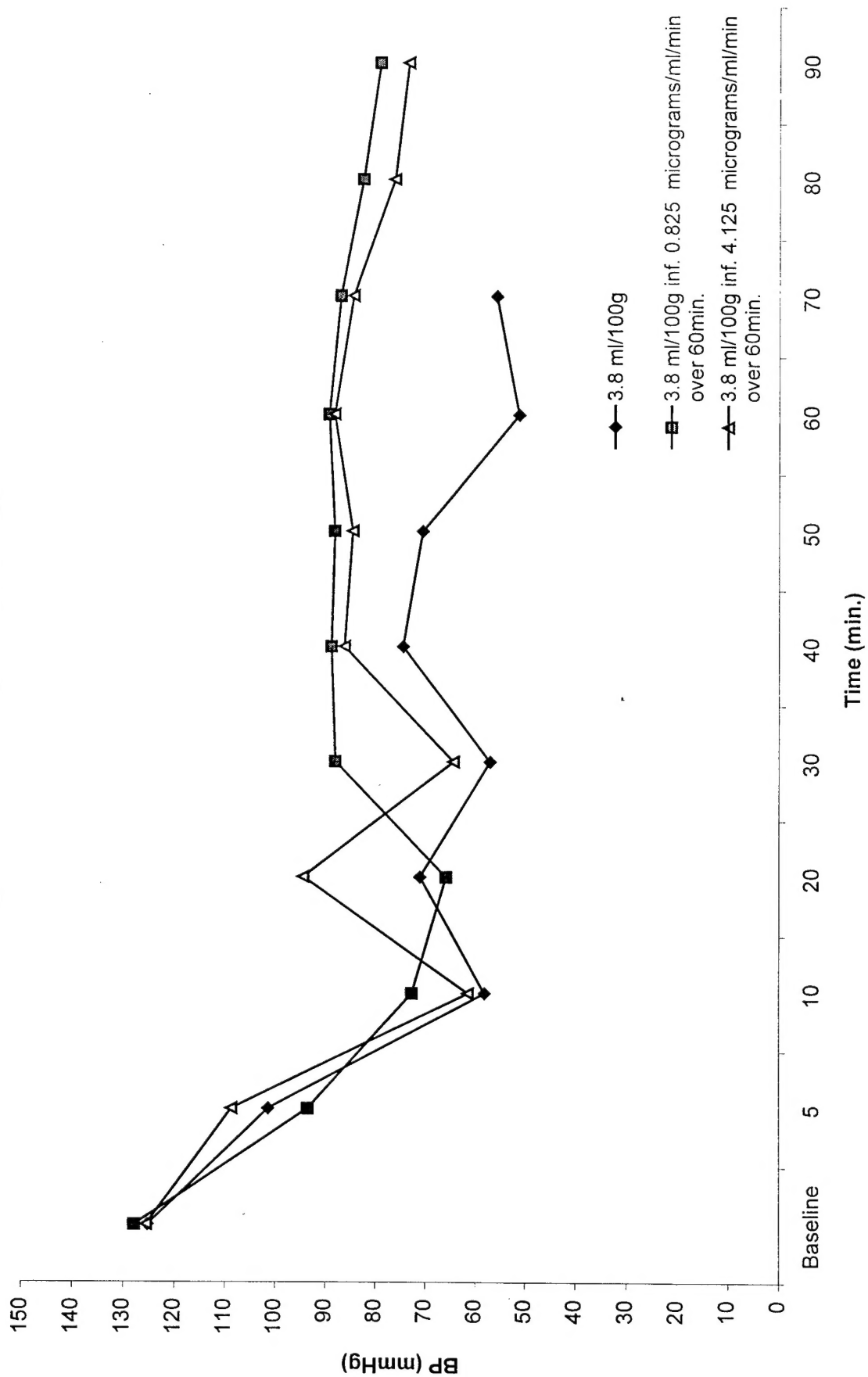


Figure 3. HR change to hemorrhage

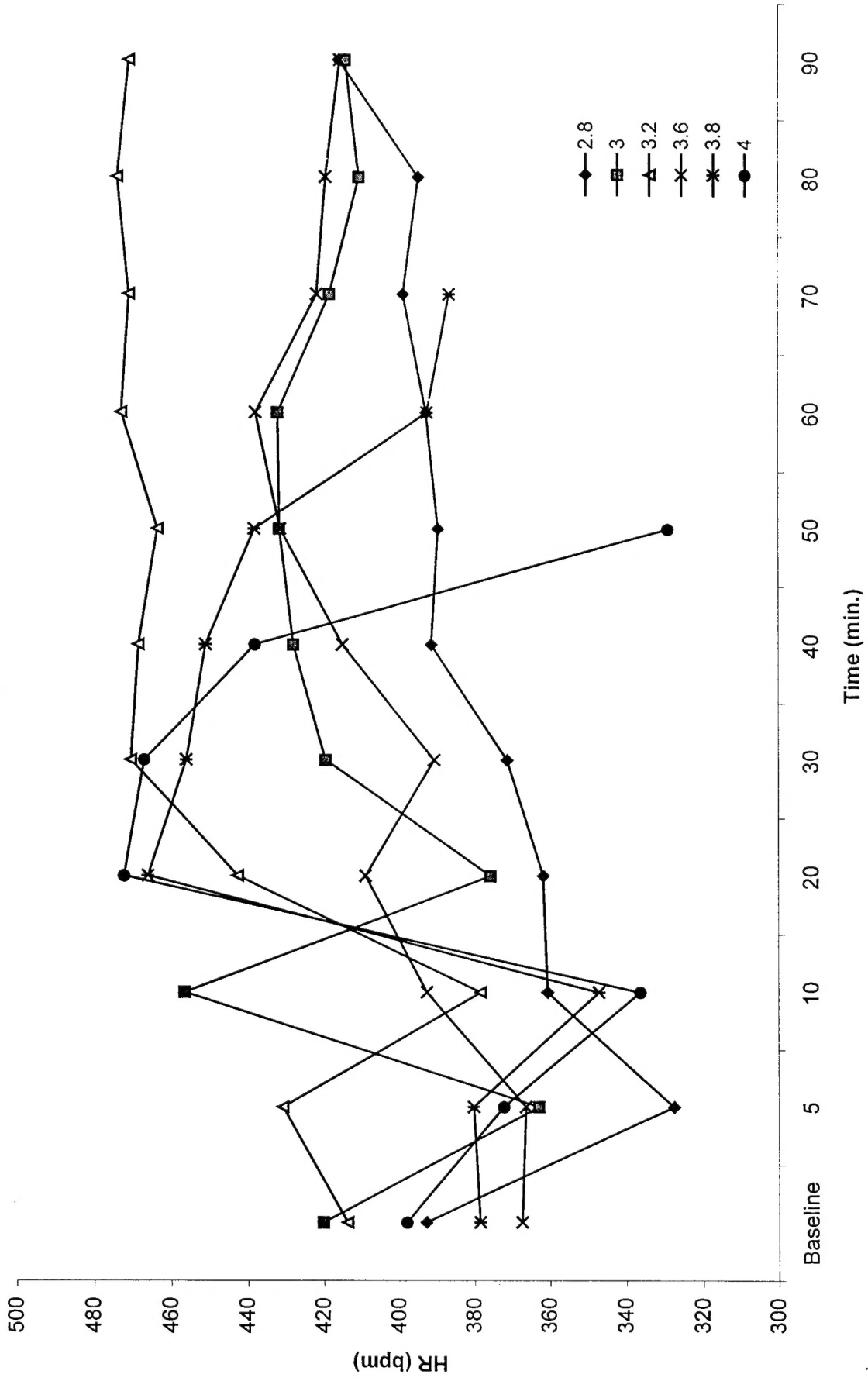




Figure 4. HR change to hemorrhage

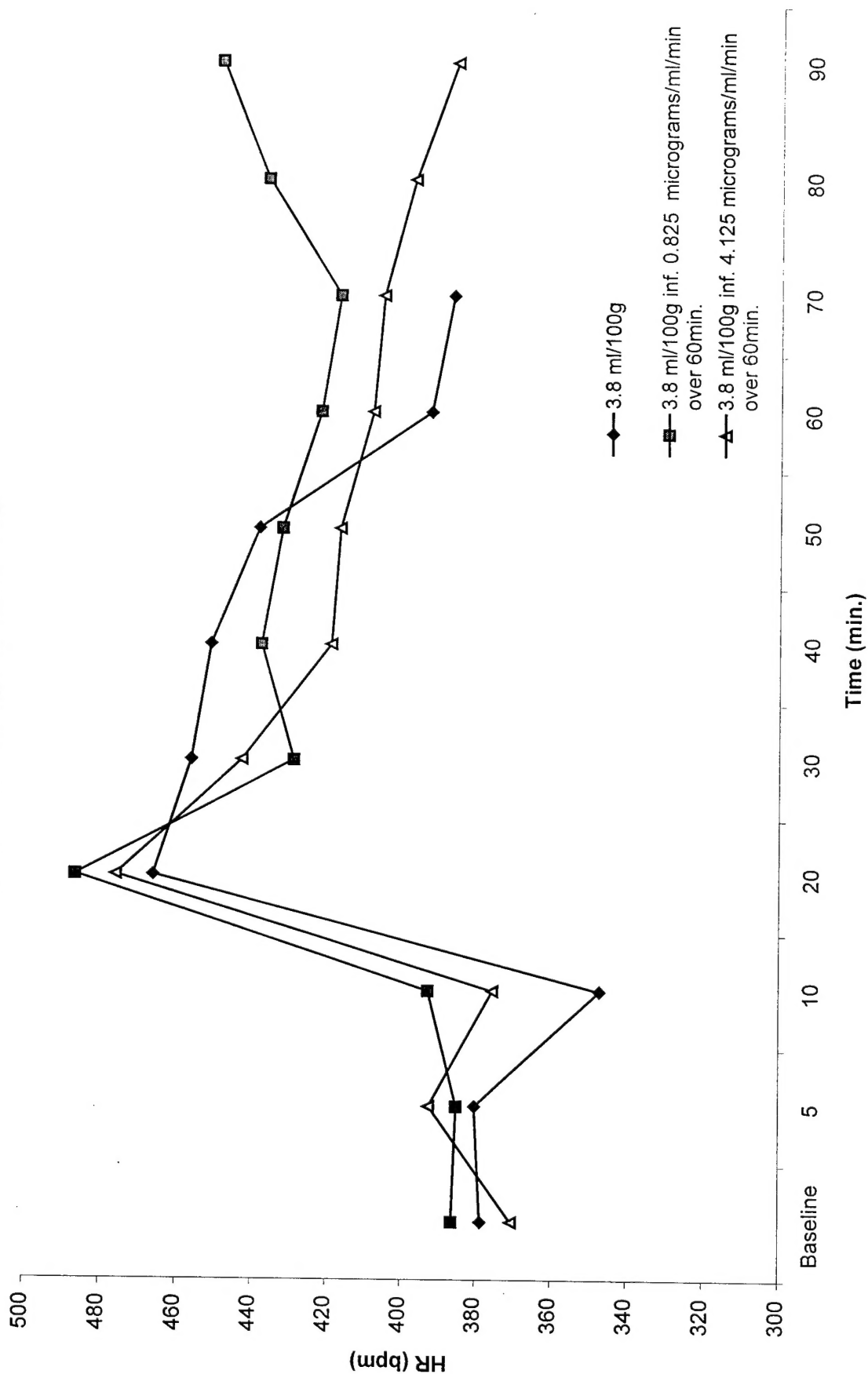
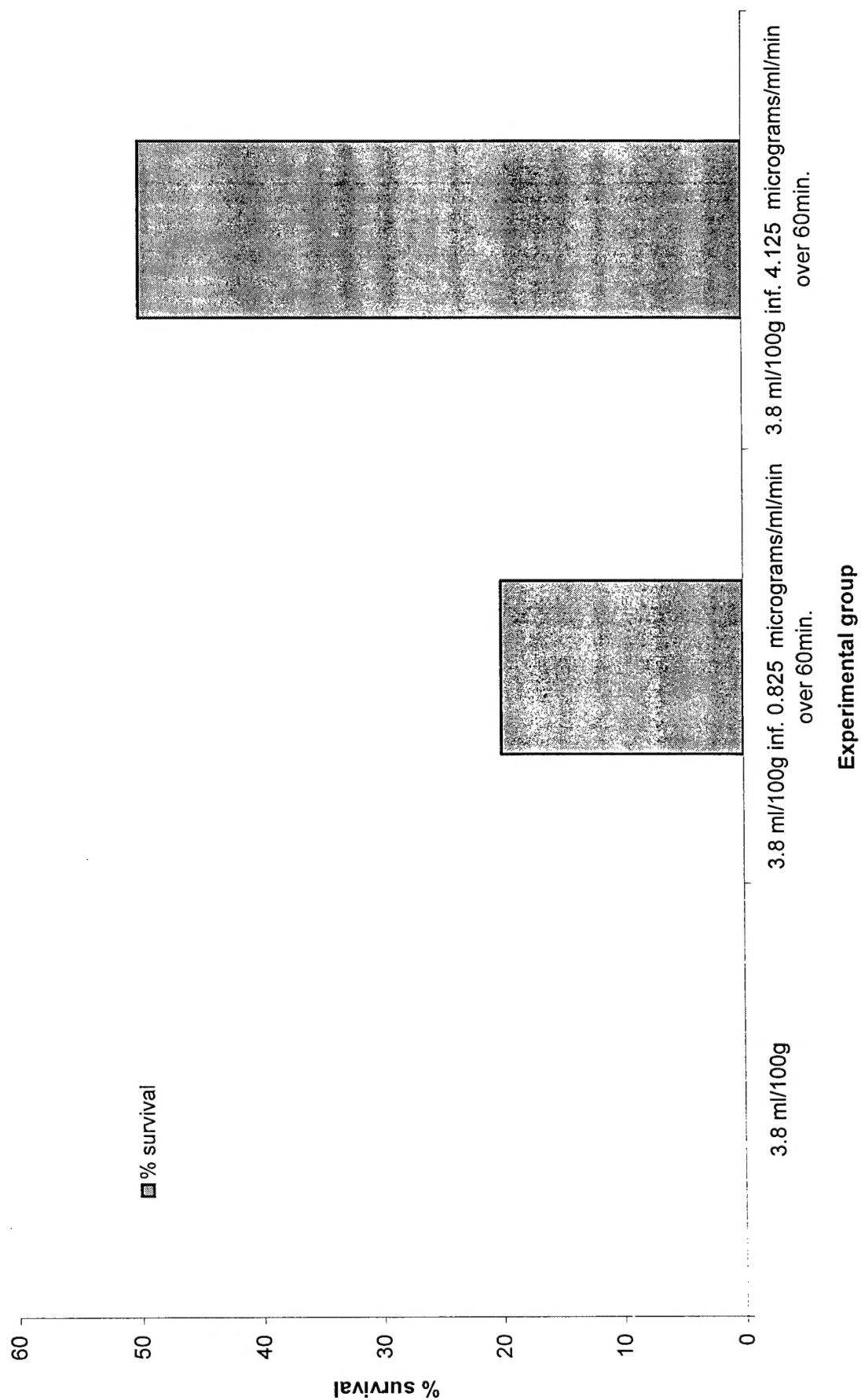


Figure 5. Survival to hemorrhage



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13. ABSTRACT (Maximum 200 words)  In response to acute blood loss in excess of 20-30% of vascular volume both human and experimental animals exhibit an abrupt withdrawal of sympathetic outflow to most vascular beds. The present research program investigates the efficacy and mechanisms of action of two treatments designed to reverse this hypovolemia-induced reduction of sympathetic tone. Specifically, $\gamma$ -melanocyte stimulatory hormone ( $\gamma$ -MSH), a melanopeptide, and systemic hypertonic saline administration are being studied for their capacity to reverse sympathetic withdrawal when administered acutely in the earliest stages of hemorrhagic shock. Over the past year, we have established a hemorrhage model in the rat that permits graded withdrawal of blood while recording critical cardiovascular parameters. Our results indicate that acute administration of $\gamma$ -MSH will raise blood pressure of severely hypotensive hemorrhaged rats. Also, initial data indicate that $\gamma$ -MSH increases the number of animals that survive severe hypotensive hemorrhage.				
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